NMR spectroscopy for Structural Biology NS
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Lecture: 11
Practical Aspects of FT-NMR-3

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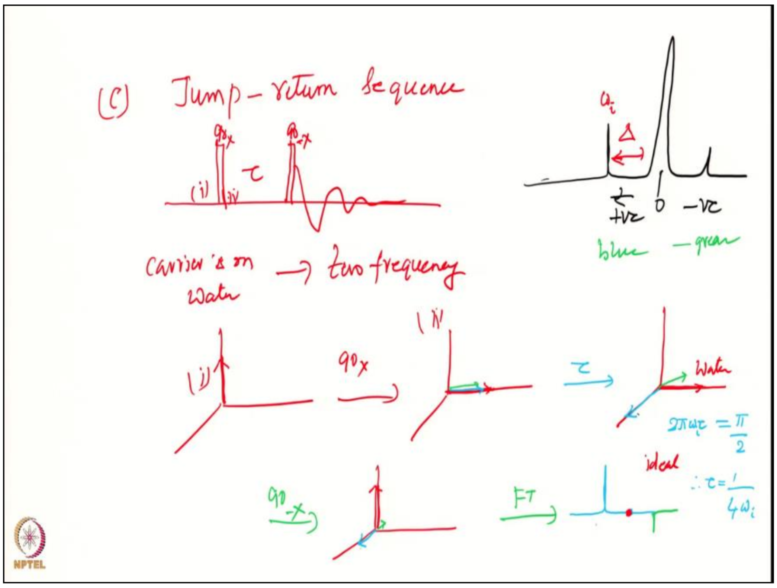

The next technique we would like to consider for water suppression is the so, called jump return. Jump return sequence the way it works is you have a 90° pulse you put the carrier on water. So, the carrier is on water wait for a time τ; then you apply 90 pulse again but this time you apply 90 - x earlier you applied a 90 x then you apply. Now a 90 - x and then you collect a FID like this.

So, how does this work let us look at let us draw the picture like this. So, initially all the magnetization is here this is at point number one this is at one and here at 2. So, apply 90x I will get let us say magnetization on to the Y axis it comes here this is at 0.2. Now different components would precess here now they will go with the different frequencies water will go with its own frequency and sample signals will also go with their own frequencies.

Now since I am sitting on the water, water does not move because there is zero frequency since I am sitting on water the water does not move. Let me say my sample signal which is present here green one during the period tau what happens the water remains there only because it does not I am sitting on the water therefore it is a zero frequency. And I will wait for the time tau such that my sample signal has come exactly here on to this.

Sample signal has come exactly on to this and the water has remained there only water has not moved because it is at zero frequency water is at zero frequency. Carrier is on water means it has zero frequency. So, that does not precess whereas your other signals will precess. So, this one signal may have come here it is also possible that there is one more signal. So, let me let me draw the spectrum here.

Suppose I have a spectrum which is like this and this is my water and this is another signal here. So, this one is positive and this is negative with respect to the water this is zero and the green the blue one which I have drawn here that has come exactly to this point. Now let me draw another colour take another one here green one and where would that go that would have moved to some other point let us say here.

Let us say this is my green and this is the blue I am using the same colour that does not matter. So, the blue one has come here and the green one has gone to this place. Now then what I do is I apply a next 90 - x. So, what will be the result of this? The result of this will be water will come back here water will go back because I am applying 90 - x I am rotating it back to the Z axis and the blue and the green components will some components will survive.

So, something will survive here of course certain other component would have gone there and similarly some component of green also will survive some component of green also will survive other components which are orthogonal to it they would also have gone back to the Z axis. Now if I do a Fourier transformation of this what will I do what will I get I will get let us say the blue one which is here I will get a positive signal like this and that the water I will get a 0.

And then I will get a green one I will get a negative the opposite side because it is gone to the other side at time t = 0 it is on the other side if this is on the plus X axis that is on the –X axis therefore the initial point it has a minus it has the opposite phase therefore it is gone here. So, this is a very ideal picture the lines of course will have this various kinds of distorted line shapes will be there but this is the ideal picture.

The water has gone back to Z axis and therefore there will be no signal of the water, ideal. In typically this does not happen ideally there will be some amount thing there will be various kinds of waste distortions will also happen the ones which are close to the water what will be the problem here which are under the water if there is a signal which is under the water you will also lose that, that also will disappear.

Because that also will go back to the Z axis you will not see. Therefore what one does is you adjust your τ such that you get maximum signal for the region of your interest. Suppose this distance is something like about delta let us say this is Δ. So, you adjust your τ such that the this has moved by this frequency has moved by 90° let me call this as your signal which is required w desired let me call this as ω_i and $2\pi\ \omega_i$ how much is the rotation of this of the blue signal.

The rotation of the blue signal here is $2\pi\ \omega_i\ \tau$ that is the rotation in angle $\omega_i\ \tau$ is in the radiance. So, in terms of θ if I want to multiply that by 2π then I get it in degrees. Now if I adjust this to $\pi/2$ that means I have made it rotate by 90° therefore what I have to do therefore I have to adjust τ_2 this, this will go away $\frac{1}{4\omega_i}$. So, by doing so, I maximize my signal for the desired signal in my spectrum. I may lose other ones it does not matter.

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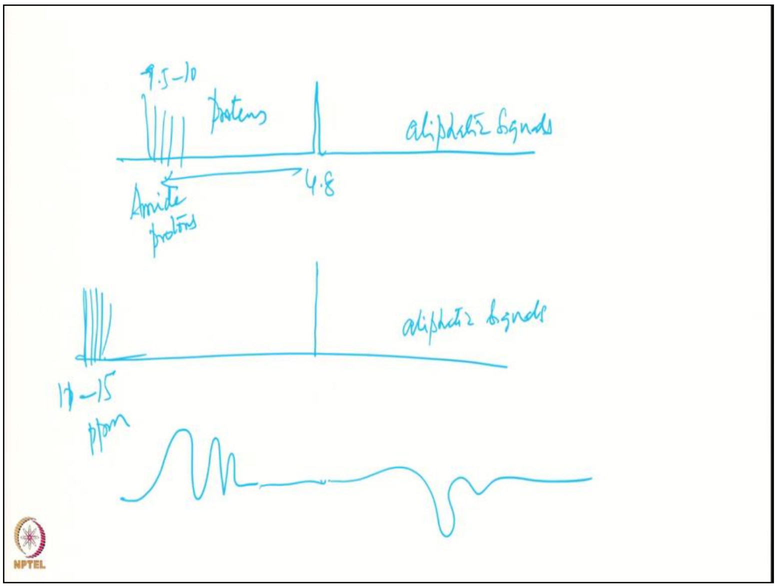

So, typically this is used in applications where you have very widely different frequencies. So, you have the water here let us say and then you have the various signals here these are the amide protons let us say. This is typically in proteins this is what you will have this water is at

4.8 ppm these are from 7.5 to 10 ppm or if you are looking at DNA. So, in DNA the water signal is here and you have the immunoprotons which are coming at 11 to 15 ppm.

And your aliphatic ones will be here aliphatic signals will be here and same is true here aliphatic signals. So, if you adjust your you keep the what keep your RF on the water and adjust your time to suit to this distance from here to here then you will get for these ones you will get positive signals on this side and then you will have the reduction here and you will get negative signals here for the aliphatic ones because these are on the opposite sides of the water.

So, this sort of water suppression is also quite efficient and has been used for quite extensive for especially for DNA this has been used very, very effectively all right. So, this is so much for the special features of FT-NMR. Now we go over to another concept which is called as spin echo.

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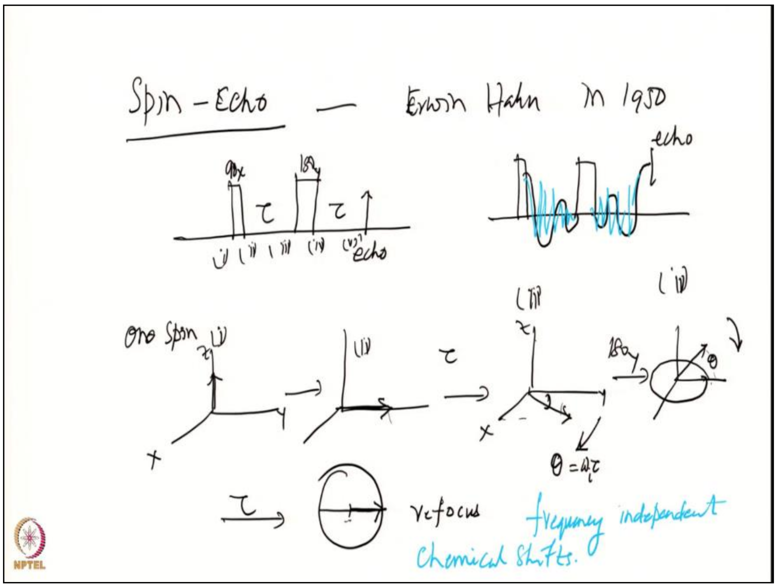

So, what is this pin echo? I mean all of you know echo what means you stand in front of a hill and shout you hear back your voice after some time the sound goes gets reflected from the hill and comes back to you that is the echo right. So, when you shout the signal has decayed and then it is gone and when it gets reflected then comes back to you then it picks up. So, that is the echo in the same manner here also you can do a magnetization which is decaying and then you recover it.

So, that you get an echo. So, that is what is the principle here. This was discovered by Hahn; Erwin Hahn in 1950 and this is one of the most important developments the discovery very simple but very elegant and very important discovery which becomes a component of all multiples experiments most multiples experiments in 2 dimension three dimension in your even in other one dimensional experiments as well.

So, what is the idea here the idea is the following. So, the pulse sequence goes like this. So, you have a 90° pulse 90x let us say your time period tau here give a time period τ then you apply 180° pulse let us say I apply it along the Y axis then the same give the same time period tau then after that what I get here. This is the echo at this point I get the echo I may collect the data afterwards but this is the echo.

What happens during this period? Let us look at it from the a single spin situation or we can do 2 spins also and let us do what happens this is the time point 1, this is time point 2 time point 3 and time point 4 and this is time point 5. So, consider one spin and let us assume that I have initial magnetization is here this is at one time point one then I apply the 90 degree pulse the magnetization has come here this is time point 2.

And then during the next period tau it has evolved with its characteristic frequency and now I will write only let me write the full thing it is rotated to some point here this is my time point 3. Now this was my x,y and z. Now what I am applying? I am applying a 180°y pulse. So, this is a 180y. So, what happens to this magnetization where does it go it goes here 180° rotation.

So, it comes goes out of the plane and comes back there to understand it much more easily you take the components along the x and the y components and you see both the components will rotate right. So, the x component will go to the –X axis y component will stay there only therefore the net result will be along this axis here. The movement is happening here, here during the period τ with the characteristic frequency its own frequency.

And how much it has moved? Let us say it is moved by an angle θ this angle theta during that period it is moved by the angle θ because it is the characteristic frequency and this is $\theta = \omega_i \tau$ it has moved by that much. Now this spin which has gone here. Now this will continue to move in the same direction once again. So, where it will come after the next time period τ this is my time point 4 where it will come after that then it will come back onto this axis I drew only the transverse plane here.

So, if we were to drop the transverse plane here this is in the transverse plane. So, this will move down here to this point it will move down to this point the Z axis is up there. So, what whatever it has moved during the period τ. So, this will again move this angle is also θ therefore it will move at the same angle θ in the next period τ. So, it will come back here. So, therefore you see this is the refocusing this is echo what was happening here during this period during the period τ.

You see the the y component was progressively decreasing right if you were to look at the FID during this period tau during the moment during the period τ what was happening the magnetization was decaying was moving right there was an FID. So, this was decaying here magnetization was decaying here right it was moving out of the Y axis. Now when it comes back here then it during the next period tau it starts coming back onto the Y axis which means it is increasing.

So, if I want to draw this here magnetization is FID is decreasing during the period tau and this is my 180° and then it starts increasing again. So, this is my echo. This is like a mountain this 180° is a mountain this is the time it takes for the signal to go here when you shout and this is the time it takes to it comes back with the same speed right the sound is the same speed it will come back here with the same speed therefore it will take the same time. So, this is the echo.

So, now you see this is regardless of what the frequency is no matter what the frequency of precision is it will come back. So, here I have drawn with one frequency suppose I took another frequency this will also come back. So, therefore this is frequency independent in other words the spin echo is said to refocus chemical shifts. So, therefore this will refocus chemical shifts. So, we are considering here a single spin and there it is completely refocusing.

If it were 2 spins also it will refocus but only thing is there should be no coupling between them. These 2 spins are not coupled if it is they are coupled then of course they do not refocus and that is a little bit more tricky and we will try and go through it appropriately.

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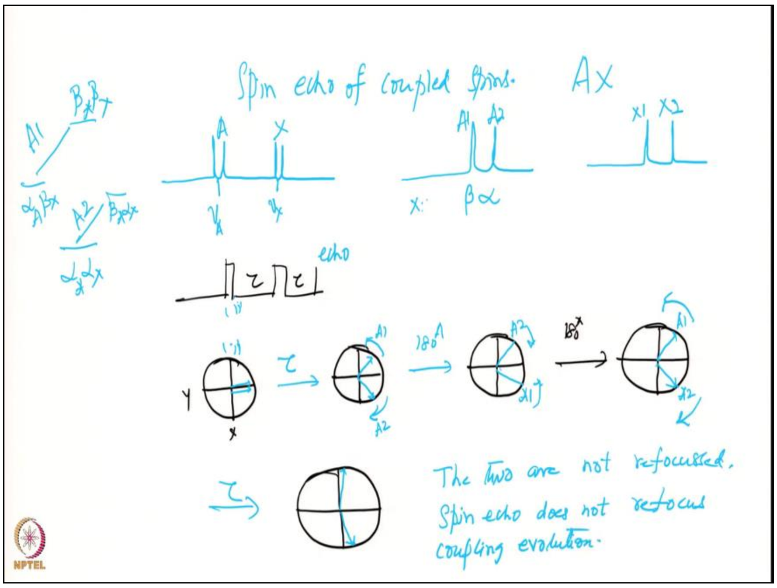

Spin echo of coupled spins, let us say I take 2 spins A and X, AX right. So, the one dimensional spectrum of this will be the A will have 2 lines and X also will have 2 lines the coupling constant between them will be the same. So, this is my A spin and this is my X spin let us say. And this is the center of the A spin and let us say this frequency is ν_A and this frequency is ν_X and the separation is the coupling constant.

Let us draw the picture for the same. Now you remember here one more thing how do these lines appear? If I want to draw the A lines little bit more elaborately here. And also the X lines somewhat more separately here properly separated and let if they I call this transition as A_1 call this as A_2 call this as X_1 and this as X_2. Now how does this transition A_1 appear you recall back what we did earlier when the transition alpha spin is flipping the A spin is flipping from alpha to beta what is the polarization of the X spin.

X spin this is in the β state and it is in the α state. For the A 2 transition this for the A_1 transition X spin is in the β state for the A_2 transition X spin is in the alpha state. So, if we can recall that in the energy level diagram there let us say this is α_A α_X $\alpha\beta$ α_A β_X β_A α_X β_A β_X. So, this I have the transition here one transition here other transition here these are my A_1 and A_2 which one is my A_1 this is my A_1 transition this is my A_2 transition.

In the A_1 transition α_A is flipping to β_A and the X spin is in the beta polarization. For the A_2 transition α is flipping to β and the X spin is in the alpha polarization that is what I have written here this is important to notice here. Now let us look at what happens when you apply the spin echo let us look at the spin echo again here. This spin echo sequence this is τ this is τ and now we sit on the in the rotating frame of the A spin.

That means we sit in the center let us that means I will want to focus only on the ace pin for the moment let us look at the movement of the A spin. So, at this point I will draw only the transverse plane. So, this is my point are both the transitions are here right at this point this is my point 2. Now they will during the next period tau what will happen these 2 transitions will go in opposite directions because I am sitting on the middle of the 2.

If I am sitting on the middle of a that means with respect to the nu A with respect to the center one way A_1 will go faster and A_2 will go slower. So, let me draw that here let us say A_1 has gone here and A_2 has gone here this is going like this, this is going like this when I apply the 180°y. Now this is these are my X and the Y axis are this is my x and this is my y. Now if I apply y pulse let me also write which is A_1, A_2 because these are all very important things.

So, this is my let us say A_1 and this is my A_2 during the next τ_p. Now apply 180° pulse let us say on to A_1 because this 180 pulse is on both right it is 180 pulses on both only pulses on both. So, what will happen during then suppose a 180 pulse on the A spin considering on the A spin. So, so this will come. So, this is a rotation like this the A_2 A_1 will come down A_2 will go here A_1 will come here.

Now how was the A_1 moving A_1 was moving like this A_2 was moving like this right. So, during the next period tau they should come back and refocus they should move with the same speed and they should come back and refocus. But now notice we are applying a pulse on the X spin as well and this is the crucial point this is the crucial point and that is what we will show here. So, in the next consider we break up this 180 pulse into 180 A and 180 X.

180x on the X spin what does it do 180 X means X spin flips from β to α, X spins flip from β to α and α to β in other words A_1 will go to A_2, A_2 will go to A_1. So, I have these 2 transition same here but now they will change their labels this will become A_1 this will become A_2 and this will continue to go this way this will continue to go this way. So, now you see there is a big difference.

So, during the next period τ what will happen they will continue to move in the same directions. This fellow would have gone there this fellow would have gone there. So, therefore they have not refocused therefore at the end of the echo this is the echo period the 2 are not refocused. So, therefore the spin echo does not refocus coupling evolution. So, what are the conclusions we have here.

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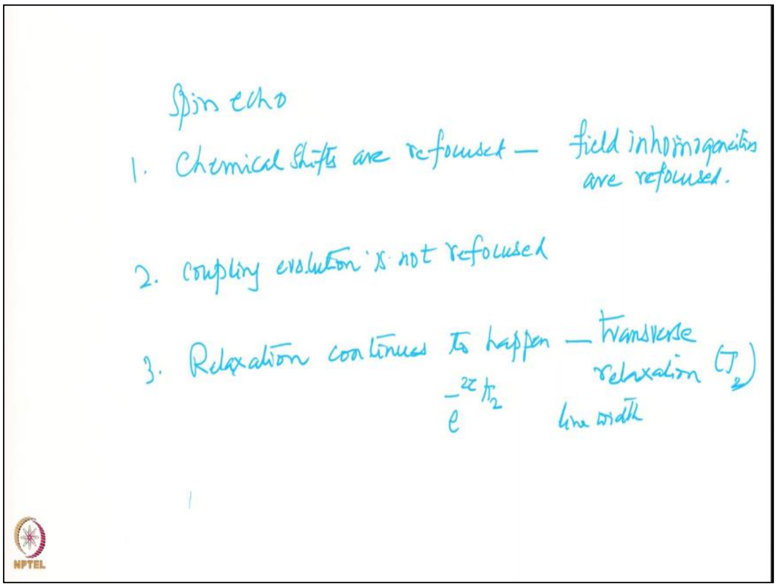

One thing is let me write that down explicitly in the separate one this spin echo 2 important points one chemical shifts are refocused this means field inhomogeneities are refocused field inhomogeneous. Because field inhomogeneous contribute to variations in the frequencies of precession right there is equivalent to chemical shift different chemical shifts field inhomogenity are refocused.

2 coupling evolution is not refocused. This is the most important part and you will see this is what is used in various other techniques in multiple experiments. The third point is relaxation continues to happen which relaxation during the spin echo relaxation continues to happen. And which relaxation is this, this is the transverse relaxation because the magnetization is in the transverse plane or T_2 relaxation.

So, therefore this provides an ideal method for the measurement of transverse relaxation times because it is not clouded by the field inhomogeneity effects. Field homogeneity when it is there they will cause dephasing because of the difference in frequencies and therefore the signal will decay faster because the lines will become broader and the field in homogeneities will sort of give a wrong impression about the T_2 relaxation that because T_2 relaxation is related to line width right.

So, this is related to line width and see if inhomogeneities are there they are contributing to your decay of the signal because of precessional frequencies then you measure a wrong line width and wrong and that cannot be taken as T_2 here the field homogeneity effects are cancelled out. So, you can use this method to obtain measure the transverse relaxation times very accurately. So, this is the important application immediate application of this of and this happens at the rate $\frac{-2\tau}{T_2}$- 2 tau by T 2.

So, this is the relaxation rate. Now there is one more point which I will take up and then we will stop. We can use this spin echo sequence for water suppression and that is water gate.

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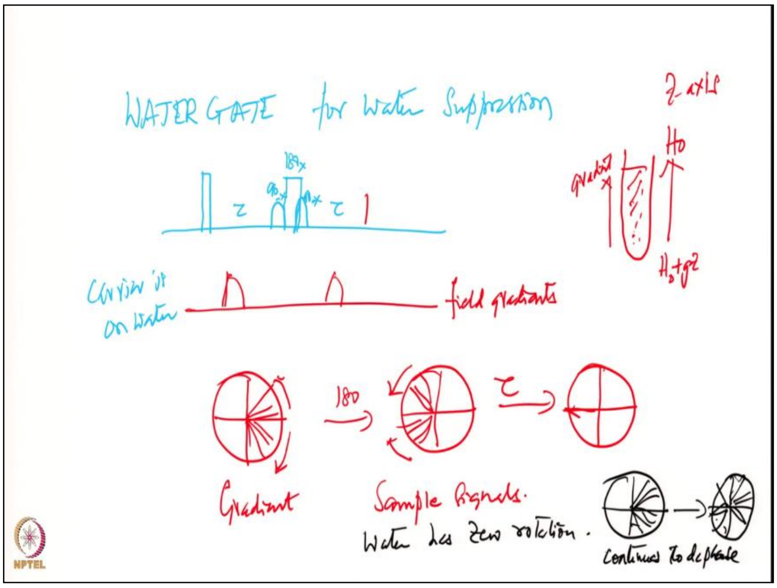

Water gate for water suppression the pulse sequence is like this. So, you apply a 90° pulse you keep on water the carrier is on water and then you apply a selective pulse 90 let us say x 90 - x then immediately you apply hard pulse 180x which is applied to all the spins and then you apply another soft pulse on water this is again a 90 - x again on water and this period τ this period τ.

But there is one more thing that happens here and that is called as the field gradients. We use here what are called as field gradients. So, you apply a field gradient here I will explain what that means you apply field gradient here when you apply field gradient here and you collect the signal from this point onwards. What does the field gradient do these are field gradients it is a linear field gradient it can be explained like this.

Suppose you have a sample your sample is here your magnetic field is in this direction there are additional coils which will change the field along the Z axis this is my Z axis it will change the field along the Z axis so, in a particular manner. So, field gradient will increase this is the gradient field that means the field at different points will be H_0 plus some coefficient times z. So, this is the gradient and this is the z coordinate along the Z axis.

Therefore this is a linear thing and g is the coefficient the rate therefore at different points the field will be different what is the meaning all those things which are seeing this they will dephase all the frequencies will become different. So, the result will be that if I draw the transverse plane here. So, all the frequencies will dephase as a result of this gradient because of the gradient.

Now if I apply 180° pulse to this and then apply the same gradient once more because these are all going in this frequent this way and this way if I apply 180° pulse then the wall would have come here. So, this will go like this, this will go like this and then during the next period town they will all come back again they will come back here. Therefore they are refocus. So, when I apply 180° pulse but now this is so, far as the sample signals are concerned.

But I have done another trick here what I have done is I have applied 180x and 90 – x, 90 –x the water sees how much flip angle the water says zero flip angle 90 –x, 90 –x is 180 –x 180 –x 180 –x is 0 therefore the water does not see any angle at all does not see any flip the water has zero rotation. So, which means after it has come down into the transverse plane these are the water signals they continue to go and after that at the end also they continue to go even more spread all over the diphase more and more.

Because they do not see the gradient effect they do not see the 180 pulse is not seen they see the gradient effect they do not see the 180 pulse they see a zero there is no inversion. Therefore they continue to deface water signal continues to dephase as a result it will all completely cancel out. So, water will be zero and at the end of this tau you will only have signals coming from the sample because they all have been refocused.

The refocusing is happening because of the 180 pulse on the sample signal and the water is seeing a zero pulse, zero rotation therefore it will continue to dephase the both the gradients will contribute to the dephasing only and therefore the water signal will completely dephase and will be eliminated and this is how you achieve water suppression in this particular scheme. So, I think we can stop here this the time is up.